Citation:

Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med.* 1999; 340: 1,449-1,454.

PubMed ID: 10320382

Study Design:

Trend study.

Class:

D - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To determine the effect of folic acid fortification on plasma total homocysteine concentration in a cohort of participants in the Framingham study.

Inclusion Criteria:

Members of the Framingham cohort in the fifth and sixth examination.

Exclusion Criteria:

- 242 subjects were excluded because they started taking folic acid supplements between the fifth and sixth examination
- 95 subjects that stopped taking supplements during this period were also excluded.

Description of Study Protocol:

Recruitment

Persons from the Framingham Offspring Cohort during the fifth and sixth examination.

Design

Cross-sectional study.

Dietary Intake/Dietary Assessment Methodology

Dietary intake of folate was determined with a food frequency questionnaire.

Statistical Analysis

- A separate analysis was completed on subjects who reported use of supplements containing folic acid from the data on those who did not. Data were log-transformed and inverse transformations were used to calculate geometric means and 95% confidence intervals adjusted for age and sex
- Low plasma folate was defined as plasma folate concentration of less than 3ng per ml (7nmol per L)
- High plasma folate was defined as plasma folate concentration greater than 13nmol per L
- Change in folate status was calculated in the study group after exposure to fortification and in the control group over a follow-up period of similar length
- Baseline and follow-up values were analyzed by SAS PROC GLM.

Data Collection Summary:

Timing of Measurements

The fifth examination of the offspring Framingham cohort, from January 1991 to December 1994, and the sixth examination, from January 1995 to August 1998.

Dependent Variables

- Plasma homocysteine, plasma folate, plasma vitamin B_{12} and pyridoxal 5'-phosphate (active form) were determined from fasting blood samples
- Dietary folate intake was assessed with a Food Frequency Questionnaire; this questionnaire also identified nutrient intake from dietary supplements and from fortified ready-to-eat breakfast cereals.

Independent Variables

- Folate fortification
- Folate status was compared between the baseline (fifth examination, pre-fortification) and the follow-up group (sixth examination, post-fortification)
- In New England, most of the targeted products were fortified with folic acid by July 1997. The sixth examination began before the start of fortification and continued until after fortification was in place. Members of the cohort whose sixth examination occurred before fortification began (January 1995 to September 1996) constituted the control group and data from the fifth and sixth examinations were used to estimate time-related changes in folate status unrelated to fortification over a three-year period.
- The study and control groups were divided into those who used vitamin supplements and those who did not.

Control Variables

Sex and age.

Description of Actual Data Sample:

- *Initial N:* 1,106 (561 males, 545 females)
- *Attrition:* 337
    - 242 were excluded due to supplement use between examinations
    - 95 were excluded due to stopping supplement use between examinations
- *Age:* 32 to 80 years
- *Location:* Framingham, MA, United States.

Summary of Results:

- Baseline plasma folate, homocysteine and B_{12} concentrations were not different between the groups. Pyridoxal 5'-phosphate concentrations were significantly lower among subjects in the study group who did not use B-vitamin supplements than among those in the control group who did not use supplements.
- Study group that did not use B-vitamin supplements: Plasma folate concentrations increased 117% after fortification (P<0.001), prevalence of low folate concentrations decreased by 92% (P<0.001), fasting total homocysteine concentrations decreased 7% (P<0.001) and the prevalence of high homocysteine concentrations decreased 48% (P<0.001) from the baseline to the follow-up
- Control group that did not take B-vitamin supplements: Significant increase in reported dietary folate intake (P<0.001)
- Study and control groups who used B-vitamin supplements: Significant increase in plasma folate concentrations from the baseline to follow-up increased 62% in the study group (P<0.001) and 24% in the control group (P<0.001). There was an 8% increase in homocysteine concentrations in the study group (P<0.006).
- At the follow-up examination, homocysteine concentrations were 10% lower among those in the study group who used supplements compared to those who did not use supplements (P<0.001), but the prevalence of high homocysteine concentrations was not significantly different between these two subgroups (P=0.62)
- The difference in homocysteine concentrations was due to differences in vitamin B_{12} and pyridoxal 5'-phosphate status between those who used B-vitamin supplements and those who did not. Plasma vitamin B_{12} concentrations were 351pg per ml (259pmol per Liter) and 475pg per ml (350pmol per Liter) for those who did and did not use supplements, respectively, (P<0.001). Pyridoxal 5'-phosphate concentrations were 53nmol and 120nmol per liter in those who did not and those that did use supplements, respectively (P<0.001). After adjustment for vitamin B_{12} and pyridoxal 5'-phosphate concentrations, the difference in homocysteine concentrations between those in the study group who used supplements and those who did not was reduced to 6% and was no longer statistically significant (P=0.10). In the study group, the prevalence of high homocysteine concentrations was essentially the same for those who used B-vitamin supplements and those who did not after adjustment for vitamin B_{12} and pyridoxal 5'-phosphate concentrations (P=0.83).

Plasma folate concentrations before and after folic acid fortification in the Framingham Offspring Study Cohort, according to the use of vitamin B supplements				
Characteristics	**No B vitamin supplements**		**B vitamin supplements**	
	Study group (N=248)	**Control group (N=553)**	**Study group (N=102)**	**Control group (N=203)**
Plasma folate-ng/ml (95%CI)				

Baseline	4.6 (4.3-4.9)	4.6 (4.4-4.8)	11.7 (10.4-13.1)	11.4 (10.5-12.4)
Follow- up	10.0 (9.3-10.7)	4.8 (4.6-5.1)	18.9 (17.0-20.9)	14.1(13.1-15.2)
Plasma folate <3 ng/ml (95%CI)				
Baseline	22.0 (17.3-26.7)	25.3 (22.1-28.4)	3.9 (0.0-11.2)	2.6 (0.0-7.7)
Follow- up	1.7 (0.0-5.4)	20.7 (18.3-23.2)	0.0 (0.0-5.9)	0.9 (0.0-5.0)
Folate intake -µg/day (95%CI)				
Baseline	266 (253-280)	275 (266-285)	650 (600-704)	651(616-689)
Follow- up	271 (258-285)	291(281-301)	686 (634-743)	675 (638-714)

Author Conclusion:

- Changes in plasma folate cannot be attributed to changes in diet, but instead were due to fortification
- The fortification had a substantial effect on plasma folate and homocysteine concentrations in a population-based sample of middle-aged and older adults
- Low folate concentrations were largely eliminated after fortification and the prevalence of high homocysteine concentrations was reduced by approximately 50% among those who did not take supplements.

Reviewer Comments:

Authors acknowledge they are unable to assess directly the effect of fortification on women of reproductive age due to the small number of women younger than 40 years, and they state: "However, we have no reason to believe that the effect of fortification on folate status in women of reproductive age differs from the effect in older adults."

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	N/A
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes

4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	N/A

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	N/A
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	Yes
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	Yes
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes

4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	No
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	Yes
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	N/A
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	Yes
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A

7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	N/A
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes